

Relationships between iodine and some trace elements in normal thyroid of males investigated by energy dispersive X-ray fluorescent analysis

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Received: 04 January 2023; **Accepted:** 12 January 2023; **Published:** 26 January 2023.

Citation: Vladimir Zaichick, (2023). Relationships between iodine and some trace elements in normal thyroid of males investigated by energy dispersive X-ray fluorescent analysis. *Journal of Clinical Oncology Reports*. 2(1). DOI: 10.58489/2836-5062/009.

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Abstract

Thyroid diseases rank second among endocrine disorders, and prevalence of the diseases is higher in the elderly as compared to the younger population. An excess or deficiency of trace element contents in thyroid play important role in goiter- and carcinogenesis of gland. The correlations with age of the seven-trace element (TE) contents (Br, Cu, Fe, I, Rb, Sr, and Zn), I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn ratios, and inter relationships between TE contents and I/TE content ratios in normal thyroid of 73 males (mean age 37.3 years, range 2.0-80) was investigated by radionuclide-induced energy dispersive X-ray fluorescent analysis. Our data reveal that the I content and the I/Fe ratio increase in the normal thyroid of male during a lifespan. Therefore, a goitrogenic and tumorigenic effect of excessive I level in the thyroid of old males and of disturbance in intrathyroidal I/Fe relationships with increasing age may be assumed. Furthermore, it was found that the levels of Br, Cu, Rb, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such TEs as Br, Cu, Rb, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Keywords: GKRS; Gamma Knife Radiosurgery, CSM; Cavernous Sinus meningioma, CNs; Cranial nerves, MIM; Multiple Intracranial Meningioma, Stereotactic Radiosurgery.

Introduction

According to the World Health Organization (WHO), thyroid diseases rank second among endocrine disorders after diabetes mellitus. More than 665 million people in the world have endemic goiter or suffer from other thyroid pathologies. At the same time, according to the same statistics, the increase in the number of thyroid diseases in the world is 5% per year [1]. It has been suggested that risk factors for the development of thyroid disorders may be numerous factors, including genetics, radiation, autoimmune diseases, as well as adverse environmental factors, such as an increase in the content of various chemicals in the environment [2].

Trace elements (TE) are among these various chemicals, because their levels in the environment have increased significantly over the past hundred

years as a result of the industrial revolution and the tremendous technological changes that have taken place in metallurgy, chemical production, electronics, agriculture, food processing and storage, cosmetics, pharmaceuticals and medicine. In connection with these changes, the levels and ratio of TE entering the human body from the outside have been significantly disturbed, compared with the conditions in which human societies have lived for many millennia.

More than 50 years ago, we formulated the postulate about the somatic TE homeostasis, which is now generally recognized [3]. According to this postulate, under evolutionary environmental conditions, the mechanisms of homeostasis of organisms maintain the levels and ratios of TE in tissues and organs within certain limits. If the content of TE in the environment changes significantly, the mechanisms

of somatic homeostasis may respond inadequately. Inadequate response of homeostasis mechanisms leads to changes in TE levels in tissues and organs, which, in turn, can affect their function and lead to the development of pathological conditions. The correctness of this conclusion was illustrated by us earlier on the example of the study of the role of TE in the normal and pathophysiology of the prostate [4-24]. It was shown, in particular, that a special role in the development of pathological transformations of the prostate is played by disturbances in the relationship between TE in the tissue and gland secretion. Moreover, it was found that changes in the relationship between TE can be used as highly informative markers of various prostate diseases, including malignant tumors [25-39]. These findings stimulated our investigations of TE relationships in thyroid tissue in normal and pathological conditions.

There are many studies regarding TE content in human thyroid, using chemical techniques and instrumental methods [40-47]. However, among the published data, no works on the relationship of TE in the normal human thyroid were found.

This work had three aims. The primary purpose of this study was to determine reliable values for the bromine (Br), copper (Cu), iron (Fe), iodine (I), rubidium (Rb), strontium (Sr), and zinc (Zn) mass fractions in the normal thyroid of subjects ranging from children to elderly males using radionuclide-induced energy-dispersive X-ray fluorescence analysis and calculate individual values of I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn. The second aim was to compare the Br, Cu, Fe, Rb, Sr, and Zn mass fractions in thyroid gland obtained in the study with published data. The final aim was to estimate the inter-correlations of TE contents and I/TE content ratios in normal thyroid of males and changes of these parameters with age.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or with comparable ethical standards.

Materials and Methods

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 73 males (European-Caucasian) aged 2.0 to 80 years. All the deceased were citizens of Obninsk and had undergone routine autopsy at the Forensic Medicine

Department of City Hospital, Obninsk. The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, or other chronic disease that could affect the normal development of the thyroid. None of the subjects were receiving medications or used any supplements known to affect thyroid TE contents. The typical causes of sudden death of most of these subjects included trauma or suicide and also acute illness (cardiac insufficiency, stroke, embolism of pulmonary artery, alcohol poisoning). All right lobes of thyroid glands were divided into two portions using a titanium scalpel [48]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the TE content determination. A histological examination was used to control the age norm conformity as well as the unavailability of microadenomatosis and latent cancer.

After the samples intended for TE analysis were weighed, they were transferred to -20°C and stored until the day of transportation in the Medical Radiological Research Center, Obninsk, where all samples were freeze-dried and homogenized [49]. To determine the contents of the TE by comparison with a known standard, aliquots of commercial, chemically pure compounds were used [50]. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 and IAEA HH-1 subsamples were prepared in the same way as the samples of dry homogenized thyroid tissue.

The TE contents in thyroid samples were determined using radionuclide-induced energy-dispersive X-ray fluorescence analysis (EDXRF) with ^{109}Cd source for Br, Cu, Fe, Rb, Sr, and Zn and ^{241}Am source for I. The mass fraction of TE was calculated by the relative way of comparing between intensities of corresponding $K\alpha$ -lines induced by radiation from radionuclide sources in tissue samples and standards. Details of the sample preparation, the facility and method of analysis were presented in our previous publication [51-53].

All thyroid samples were prepared in duplicate, and mean values of TE contents were used in final calculation. Using Microsoft Office Excel, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for TE contents and I/TE content ratios. Pearson's correlation coefficient was used in Microsoft Office Excel to calculate the relationship "age – TE mass fraction", as well as to

identify relationships between different TE contents and between different TE content ratios.

Results

Table 1 depicts comparison of our data for seven TE in ten sub-samples of CRM IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) with the corresponding certified values of TE contents in these materials.

Table 1: EDXRF data of Br, Cu, Fe, I, Rb, Sr, and Zn contents in certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) compared to certified values (mg/kg, dry mass basis)

Element	IAEA H-4 animal muscle	This work results	IAEA HH-1 human hair	This work results
Br	4.1±1.1a	5.0±0.9	4.2±2.1b	3.9±1.6
Cu	4.0±1.0a	3.9±1.1	10.2±3.2a	-
Fe	49.1±6.5a	47.0±1.0	23.7±3.1a	25.1±4.3
I	0.08±0.10b	<1.0	20.3±8.9b	19.1±6.2
Rb	18.7±3.5a	22±4	0.94±0.09b	0.89±0.17
Sr	-	<1	0.82±0.16b	1.24±0.57
Zn	86.3±11.5a	91±2	174±9a	173±17

M – arithmetical mean, SD – standard deviation, a – certified values, b – information values.

Table 2 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Br, Cu,

Fe, I, Rb, Sr, and Zn mass fractions, as well as I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in normal thyroid of male.

Table 2: Some statistical parameters of Br, Cu, Fe, I, Rb, Sr, and Zn mass fraction (mg/kg, dry tissue) as well as I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in normal thyroid of male (n=73).

Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Br	10.8	10.0	1.3	1.90	54.4	8.05	2.33	42.0
Cu	4.25	1.48	0.20	1.10	7.50	4.15	1.78	7.39
Fe	221	102	13	47.1	502	224	58.4	419
I	1487	902	130	220	3744	1337	222	3443
Rb	10.05	6.96	0.89	1.80	42.9	8.60	2.65	27.5
Sr	4.52	3.27	0.43	0.10	13.7	3.55	0.44	12.4
Zn	122	40.9	5.2	35.4	221	115	57.2	201
I/Br	226	183	24	4.26	902	191	13.1	645
I/Cu	425	393	57	30.9	2055	312	34.8	1363
I/Fe	11.0	12.5	1.7	0.223	59.4	5.97	0.749	41.3
I/Rb	221	229	31	11.1	1036	162	19.1	839
I/Sr	1046	2484	351	13.2	16570	390	49.8	5353
I/Zn	14.0	9.0	1.2	0.679	36.7	12.0	1.43	27.3

Mean – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, P 0.025 – percentile with 0.025 level, P 0.975 – percentile with 0.975 level.

The comparison of our results with published data for the Br, Cu, Fe, I, Rb, Sr, and Zn contents in the human

thyroid is shown in Table 3.

Table 3: Median, minimum and maximum value of means Br, Cu, Fe, I, Rb, Sr, and Zn contents in normal human thyroid according to data from the literature in comparison with our results (mg/kg, dry tissue).

Element	Published ta [Reference]			This work
	Median of means	Minimum of means	Maximum of means	N=105
	(n)*	M or M±SD, (n)**	M or M±SD, (n)**	M±SD
Br	18.1 (11)	5.12 (44) [40]	284±44 (14) [41]	10.8±10.0
Cu	6.1 (57)	1.42 (120) [42]	220±22 (10) [43]	4.25±1.48
Fe	252 (21)	56 (120) [42]	2444±700 (14) [41]	221±102
I	1888 (95)	159±8 (23) [44]	5772±2708 (50) [45]	1487±902
Rb	12.3 (9)	≤0.85 (29) [46]	294±191 (14) [41]	10.1±6.96
Sr	0.73 (9)	0.55±0.26 (21) [47]	46.8±4.8 (4) [43]	4.53±3.27
Zn	118 (51)	32 (120) [42]	820±204 (14) [41]	122±41

M – arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** – number of samples.

To estimate the effect of age on the TE contents and I/TE content ratios Pearson's correlation coefficient was used (Table 4).

Table 4: Correlations between age (years) and trace element content (mg/kg, dry tissue), as well as between age and I/trace element mass fraction ratios in the normal thyroid of males (*r* – coefficient of correlation)

Element	Br	Cu	Fe	I	Rb	Sr	Zn
<i>r</i>	0.13	0.22	-0.17	0.34 ^b	0.01	0.03	0.14
Ratio	I/Br	I/Cu	I/Fe	I/Rb	I/Sr	I/Zn	-
<i>r</i>	0.13	0.21	0.32 ^a	0.18	0.08	0.09	-

Statistically significant values: a $p \leq 0.05$, b $p \leq 0.01$.

The data of inter-correlation calculations (values of *r* – Pearson's coefficient of correlation) including all TE and I/TE ratios identified by us are presented in Tables 5 and 6, respectively.

Discussion

Good agreement of the Br, Cu, Fe, I, Rb, Sr, and Zn contents analyzed by EDXRF with the certified data of CRMs IAEA H-4 and IAEA HH-1 (Table 1) indicates an acceptable accuracy of the results obtained in the study for TE contents and I/TE content ratios in the normal male thyroid presented in Tables 2–6.

The content of TE was determined in all or most of the examined samples, which made it possible to calculate the main statistical parameters: the mean value of the mass fraction (M), standard deviation (SD), standard error of the mean (SEM), minimum (Min), maximum (Max), median (Med), and percentiles with levels of 0.025 (P 0.025) and 0.975 (P 0.975), of the Br, Cu, Fe, I, Rb, Sr, and Zn mass fractions, as well as I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in normal thyroid of males (Table 2). The values of M, SD, and SEM can be used to compare data for different groups of samples only

under the condition of a normal distribution of the results of determining the content of TE in the samples under study. Statistically reliable identification of the law of distribution of results requires large sample sizes, usually several hundred samples, and therefore is rarely used in biomedical research. In the conducted study, we could not prove or disprove the “normality” of the distribution of the results obtained due to the insufficient number of samples studied. Therefore, in addition to the M, SD, and SEM values, such statistical characteristics as Med, range (Min-Max) and percentiles P 0.025 and P 0.975 were calculated, which are valid for any law of distribution of the results of TE content in thyroid tissue.

The obtained means for Br, Cu, Fe, Rb, Sr, and Zn mass fraction, as shown in Table 3, agree well with the medians of mean values cited by other researches for the human thyroid, including samples received from person

s who died from different non-thyroid diseases [40–47]. A number of values for TE mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these

values using published data for water (75%) [54] and ash (4.16% on dry mass basis) [55] contents in thyroid of adults. No published data referring to I/Br, I/Cu, I/Fe, I/Rb, I/Sr, and I/Zn mass fraction ratios in human thyroid was found.

With age, the I content and the I/Fe ratio increase (Table 4). These characteristics can be used to estimate the "biological age" of the male thyroid gland.

A significant direct correlation between the Br and Cu, Br and Rb, Br and Sr, Br and Zn, Cu and Fe, Cu and Rb, Rb and Cu mass fractions as well as an inverse correlation between I and Fe mass fractions was seen in male thyroid (Table 5). Since no correlations were

found between I and other TE, except for an inverse correlation between I and Fe, it would appear that the content of Br, Cu, Rb, Sr, and Zn in the thyroid gland is independent of I content. However, this is not quite so. If we bring the content of the studied TE to the content of I (I/TE ratio), then there are close relationships between I/Br, I/Cu, I/Fe, I/Rb, and I/Zn. (Table 6). From this it follows that, at least, the levels of Br, Cu, Fe, Rb, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such TE as Br, Cu, Fe, Rb, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Table 5: Intercorrelations of the trace element mass fractions in the normal thyroid of male (r – coefficient of correlation)

Element	Br	Cu	Fe	I	Rb	Sr	Zn
Br	1.00	0.29a	0.15	0.03	0.30a	0.30a	0.41b
Cu	0.29a	1.00	0.41b	-0.07	0.27a	-0.15	0.22
Fe	0.15	0.41b	1.00	-0.33	0.20	0.07	0.13
I	0.03	-0.07	-0.33	1.00	-0.05	-0.12	0.01
Rb	0.30a	0.27a	0.20	-0.05	1.00	0.11	0.10
Sr	0.30a	-0.15	0.07	-0.12	0.11	1.00	0.17
Zn	0.41b	0.22	0.13	0.01	0.10	0.17	1.00

Statistically significant values: a $p \leq 0.05$, b $p \leq 0.01$.

Table 6: Intercorrelations of the I/trace element mass fraction ratios in the normal thyroid of male (r – coefficient of correlation)

Ratio	I/Br	I/Cu	I/Fe	I/Rb	I/Sr	I/Zn
I/Br	1.00	0.50c	0.41b	0.48c	0.26a	0.59c
I/Cu	0.50c	1.00	0.64c	0.73c	0.14	0.64c
I/Fe	0.41b	0.64c	1.00	0.55c	0.15	0.69c
I/Rb	0.48c	0.73c	0.55c	1.00	0.06	0.60c
I/Sr	0.26a	0.14	0.15	0.06	1.00	0.17
I/Zn	0.59c	0.64c	0.69c	0.60c	0.17	1.00

Statistically significant values: a $p \leq 0.05$, b $p \leq 0.01$, c $p \leq 0.001$.

Conclusion

The ^{109}Cd and ^{241}Am radionuclide-induced energy-dispersive X-ray fluorescence analysis is a useful analytical tool for the non-destructive determination of TE contents in the thyroid tissue samples. This method allows determine means for Br, Cu, Fe, I, Rb, Sr, and Zn (seven TE).

Our data reveal that the I content and the I/Fe ratio increase in the normal thyroid of male during a lifespan. Therefore, a goitrogenic and tumorigenic effect of excessive I level in the thyroid of old males

and of disturbance in intrathyroidal I/Fe relationships with increasing age may be assumed. Furthermore, it was found that the levels of Br, Cu, Rb, and Zn in the thyroid gland are interconnected and depend on the content of I in it. Because I plays a decisive role in the function of the thyroid gland, the data obtained allow us to conclude that, along with I, such TEs as Br, Cu, Rb, and Zn, if not directly, then indirectly, are involved in the process of thyroid hormone synthesis.

Acknowledgements

We are grateful to Dr. Yu. Choporov, Head of the

Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.

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